Steroidal Sapogenins. XXXVI. Side Chain Bromination Isomers of Diosgenin and Tigogenin[2]

BY MONROE E. WALL AND HOWARD W. JONES

Bromination of diosgenin acetate with 3 moles of bromine in acetic acid solution for 2 to 3 hr. gave a mixture of 5α,6β,23a-tribromodiosgenin acetate (I) and the corresponding 23b isomer II which could be separated by fractional crystallization. Treatment of I with sodium iodide in ethanol gave 23a-bromodiosgenin acetate (III). Similar treatment of II yielded 23b-bromodiosgenin acetate (IV). Catalytic hydrogenation of III and IV gave, respectively, 23a-bromotigogenin acetate (V) and 23b-bromotigogenin acetate (VI). V and VI were also obtained by monobromination of tigogenin acetate. Prolonged treatment of diosgenin with $3^1/_2$ to $3^1/_2$ moles of bromine gave two isomeric tetrabromide isomers, arbitrarily designated tetrabromodiosgenin acetates A (VII) and B (VIII). Treatment of VII and VIII with sodium iodide gave the same dibromodiosgenin acetate (IX) which on catalytic hydrogenation gave dibromotigogenin acetate (X). Bromination of tigogenin acetate gave two dibromotigogenin acetate isomers, A (X) and B (XI). Dibromotigogenin acetate A was identical with the product obtained by hydrogenation of dibromodiosgenin acetate IX. Treatment of X and XI with sodium iodide in ethanol resulted in conversion of XI to X.

Bromination of the steroidal sapogenin side chain has received sporadic attention in the past 15 years. Marker and Rohrmann,[3] who first investigated the problem, stated that only one bromine atom could be introduced in the side chain of 25D ("iso") or 25L ("normal") sapogenins. Later Djerassi[4] and co-workers found that two atoms of bromine could be introduced in the side chain of sarsasapogenin (25L series). More recently Ziegler, Rosen and Shabica[5] have found that smilagenin (25D) series) can also be dibrominated in the side chain. In addition there have been several reports of the existence of bromine side chain isomers.[6] Recently Barton, Page and Shoppee[7] have on the basis of infrared spectra in the 700–500 cm.$^{-1}$ region assigned the equatorial position to the 23a compounds of the type described by Mueller and Norton[6a] and Dickson and Page.[6b] The 23b isomers were accordingly designated axial. The location of the bromine in the various side-chain brominated sapogenins has been placed at C_{23}[8] and accepted by subsequent workers. Although this assignment is logical, since the bromine atoms are located on a carbon atom adjacent to a potential carbonyl, it must be emphasized that the C_{23} assignment is not based on a rigid structure proof.

The experiments to be reported in this paper were the outgrowth of our interest in the stereochemistry of the spiroketal side chain.[9] On bromination of diosgenin acetate with 3 moles of bromine in acetic acid at 15–20°, a precipitate was noted. After filtration, the products in both the precipitate and filtrate fractions were isolated. It was apparent from the properties of the two fractions that they were isomeric. Since the infrared spectra of the soluble and insoluble fractions resembled, respectively, those of the 23a and 23b series described by Dickson and Page,[6b] we designated the acetic acid soluble fraction as 5α,6β,23a-tribromodiosgenin acetate (I) and the insoluble, precipitate fraction as 5α,6β,23b-tribromodiosgenin acetate (II). The structural assignments of I and II were on the following evidence. Bromine analysis showed that three bromine atoms were present. The infrared absorption bands between 800–840 cm.$^{-1}$ characteristic of the Δ^5-ethylenic moiety in diosgenin[10] and

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(2) Presented in part at the Delaware Valley Regional Meeting, American Chemical Society, February 16, 1956. Paper XXXV in this series, to *J. Org. Chem.*, in press.

(3) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 846, 1516 (1939).

(4) C. Djerassi, H. Martinez and G. Rosenkranz, *J. Org. Chem.*, **16**, 303 (1951).

(5) J. B. Ziegler, W. E. Rosen and A. C. Shabica, THIS JOURNAL, **77**, 1223 (1955).

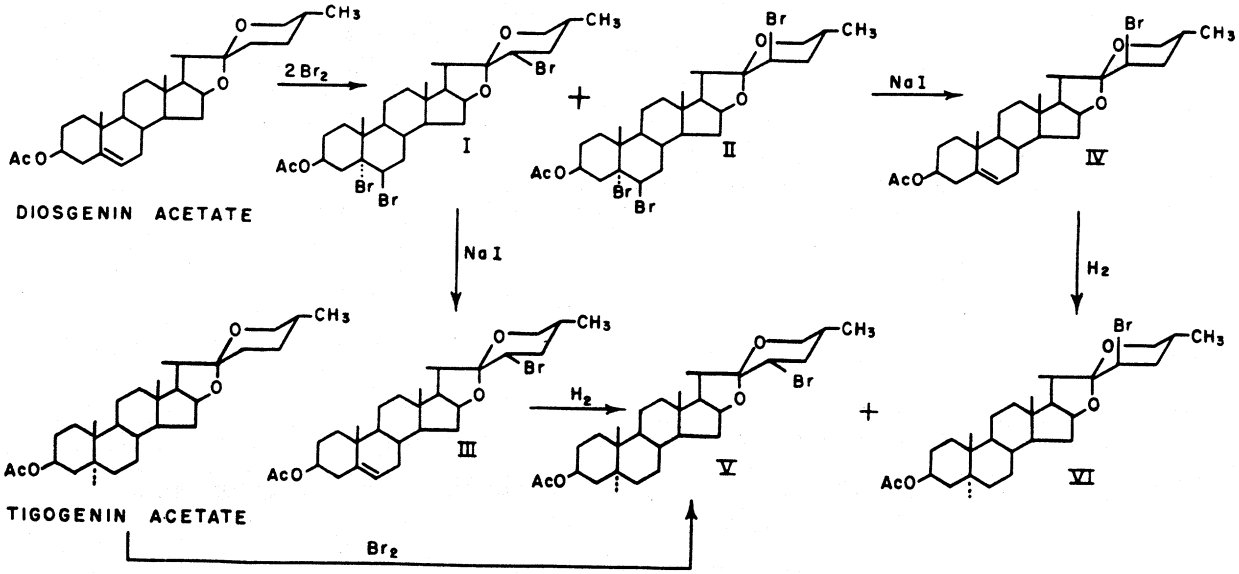
(6) (a) G. P. Mueller and L. L. Norton, *ibid.*, **76**, 749 (1954); (b) D. H. W. Dickson and J. E. Page, *J. Chem. Soc.*, 447 (1955).

(7) D. H. R. Barton, J. E. Page and C. W. Shoppee, *ibid.*, 331 (1956).

(8) R. E. Marker, D. L. Turner, A. C. Shabica and P. R. Ulshafer, THIS JOURNAL, **63**, 1032 (1941).

(9) Leading references on this subject can be found in a review by M. E. Wall, *Experientia*, **11**, 340 (1955).

(10) C. R. Eddy, M. E. Wall and M. K. Scott, *Anal. Chem.*, **25**, 266 (1953).



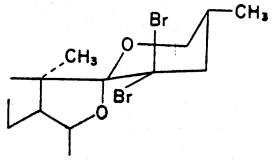
other steroids[11] were absent. It is reasonable to conclude therefore that two bromine atoms were located at carbons 5 and 6. By analogy with the bromination of cholesterol, the structure should be $5\alpha,6\beta$.[12] The assignment of two bromine atoms in the $5\alpha,6\beta$ location was further strengthened by the finding that I and II on refluxing with sodium iodide in ethanol were smoothly converted to 23a-bromodiosgenin acetate (III) and 23b-bromodiosgenin acetate (IV). Compounds III and IV on analysis showed only one bromine atom. The infrared spectra showed that the Δ^5-moiety had been regenerated. Since only the bromine atoms in the diaxial $5\alpha,6\beta$ grouping are smoothly eliminated by sodium iodide,[12a] the structural assignments of I and II at carbons 5 and 6 seem justified. Treatment of I, II, III and IV with zinc–acetic acid removed the bromine and in all cases gave the original diosgenin acetate. Bromination of tigogenin acetate with 1.5 moles of bromine under the previously described conditions gave a mixture of 23a-bromotigogenin acetate (V) and 23b-bromotigogenin acetate (VI). The mixture could be separated partially by chromatography, and final purification was achieved by fractional crystallization. Compounds V and VI analyzed for one bromine atom. The infrared spectra showed the typical 23a and 23b-bromosapogenin bands. Catalytic hydrogenation of III and IV with platinum oxide (Adams catalyst) in ether containing 5% acetic gave the corresponding 23a- (V) and 23b-bromotigogenin acetate (VI). Treatment of V and VI with zinc–acetic acid gave tigogenin acetate.

Introduction of one bromine atom into the sapogenin side chain proceeded smoothly with both diosgenin and tigogenin acetates, yields averaging about 65–75%. The 23a bromo isomers were always the predominant by a large factor, although the ratio of 23a to 23b could not be determined exactly because of losses incurred during the separation of the isomers.

(11) R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

(12) (a) D. H. R. Barton and E. Miller, THIS JOURNAL, **72**, 1066 (1950); (b) J. B. Ziegler and A. C. Shabica, *ibid.*, **74**, 4891 (1952).

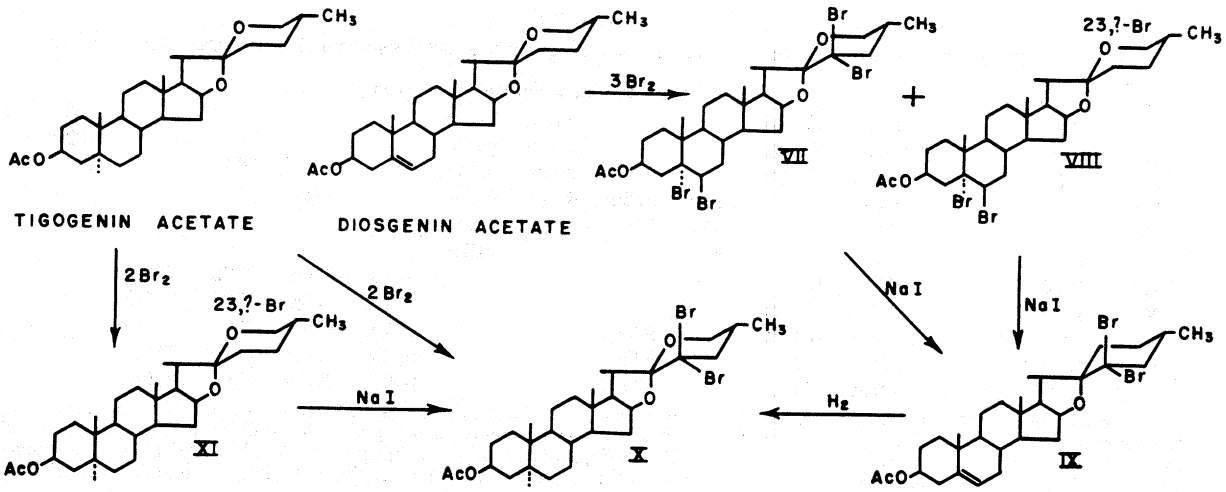
The problem of the stereochemistry of the 23a- and 23b-bromosapogenins is of interest. The partial formulation shown below represents a conception of the 25D type of bromosteroids. The configuration of ring F has been accepted by a number of groups.[9] It is assumed that the bromine atoms are located at C-23. Inspection of Fisher–Hirsch-



felder models constructed as formulated above would indicate little stereochemical preference for either the 23a- or 23b-isomers. If the mechanism of bromination of the sapogenin side chain bears any relationship or analogy to that of α-brominated ketones, then the axial epimer should be the product of kinetic control and formed first. The most stable epimer (which in this case could be either axial or equatorial) would be the product of thermodynamic control.[13] We attempted to determine which isomer was the most stable by equilibrating 23a-bromodiosgenin and -tigogenin acetates and the corresponding 23b-epimers with hydrogen bromide in ether or glacial acetic acid. In all cases the starting materials were recovered *unchanged*. In another experiment the two isomeric 23-bromodiosgenin acetates III and IV were treated with 2 moles of bromine for 5 hr. Only the corresponding 23a- and 23b-tribromides I and II could be isolated. Thus the methodology applicable to the prediction of the stereochemistry of α-bromoketones is not applicable to the bromosapogenin side chain. Another attempt to determine the stereochemistry of the 23a- and 23b-epimers involved the use of deuterium. Corey, *et al.*,[14] had shown that there was a difference in the C–D stretching absorption between equatorial and axial

(13) E. J. Corey, *Experientia*, **9**, 329 (1953).

(14) E. J. Corey, R. A. Sneen, M. G. Danaker, R. L. Young and R. L. Rutledge, *Chem. and Ind.*, 1294 (1954).



deuterium epimers. We treated 23a- and 23b-bromodiosgenin acetates with zinc dust and *o*-deuteroacetic acid. However, we obtained the same deuterated compound in both instances. Callow, James and Massy–Beresford[15] have shown that the same reaction takes place with tigogenin bromide isomers and that deuterium will also replace two hydrogen atoms in tigogenin acetate (presumably at C_{23}) when the latter is refluxed with deuterium actate.[16] It seems likely that the replaceable hydrogen atoms are located at C_{23} adjacent to a potential carbonyl at C_{22}. We found that treatment of diosgenin acetate with deuterium acetate or refluxing 23a- and 23b-bromodiosgenin acetates with zinc–deuterium acetate all gave the same deuterated diosgenin acetate. This indicates that the replaced hydrogen atoms must be labile. The corresponding deuterium atoms are also labile. Bromination of the deuterated diosgenin acetate gave a tribromo compound in which deuterium was absent. Similarly treatment of the former with acetic anhydride–pyridine hydrochloride gave a pseudodiosgenin acetate with no deuterium. It is apparent therefore that the deuteration procedure of Corey[14] cannot be applied to determination of the conformation of the bromine atoms at C_{23} in the sapogenin side chain.

Recently Barton, Page and Shoppee studied the infrared spectra of epimeric pairs of halogenated steroids of known conformation. They found that steroids with an equatorial carbon–halogen linkage invariably had C–halogen bands absorbing at a higher frequency than the corresponding axial epimer. Similar differences were noted between epimeric 23a- and 23b-bromosapogenins in the 25D series. We have confirmed these findings with 23a-bromodiosgenin acetate and its 23b-epimer. Barton, *et al.*,[7] concluded that if the bromine atoms were in fact attached to C_{23}, the 23a- type was equatorial and the 23b- axial. We feel that this hypothesis is attractive but would prefer to have more conclusive chemical evidence on this subject.

Turning next to the subject of dibromination of the spiroketal side chain, it will be recalled that conducting the bromination in acetic acid with a large excess of bromine for time periods up to 2 hr. did not lead to the introduction of a second atom of bromine.[17] Changing the solvent to chloroform, benzene, carbon tetrachloride or methylene chloride had no effect, nor did use of anhydrous glacial acetic acid. However, when diosgenin acetate was treated with $3^1/_3$–$3^1/_2$ moles of bromine in acetic acid at room temperature and allowed to stand for 72 hr., a poor yield of tetrabromodiosgenin acetate was obtained. Most of the material was resinous. Chromatography of the tetrabromodiosgenin acetate gave a major fraction (VII) which was eluted in hexane and a minor product (VIII), eluted with benzene. Bromine analysis showed that both VII and VIII had four bromine atoms. The infrared spectra of the two isomers were markedly different. On refluxing VII and VIII with sodium iodide–ethanol, the same dibromodiosgenin acetate (IX) was obtained. Catalytic hydrogenation of IX gave dibromotigogenin acetate (X).

Bromination of tigogenin acetate under similar conditions gave a low yield of crystalline dibromotigogenin acetate. Chromatography gave a major fraction identical to X above and a minor isomer XI. The infrared spectra of X and XI differed characteristically and were similar to the spectra of VII and VIII, respectively. Compound X was not affected by sodium iodide–ethanol whereas XI was converted to X. Neither X nor XI was affected by treatment with hydrogen bromide in glacial acetic acid. From the data it is apparent that VII, IX and X have bromine atoms in the same location in the side chain. We have tentatively assigned the 23,23-dibromo- configuration to these compounds. The unstable compounds VIII and XI obviously must have one bromine atom located in some position other than C_{23}. Attempts to

(15) R. K. Callow, V. H. T. James and P. N. Massy–Beresford, *Chem. and Ind.*, R26 (1956).

(16) We conducted the deuteration experiments on the isomeric diosgenin bromides prior to the publication of reference 15. Shortly after we carried out this experiment we had the pleasure of an interesting discussion with Dr. Callow at which time he was kind enough to give us information in advance of the publication of reference 15.

(17) The replacement of the first hydrogen atom by bromine proceeds rapidly, the rate being comparable to the addition of bromine to the Δ^5-ethylenic bond. This is indicated by the fact that addition of one mole of bromine to diosgenin acetate invariably gave mixtures which were partially side chain brominated.

TABLE I

CHARACTERISTIC FREQUENCIES OF DIOSGENIN AND TIGOGENIN ACETATES WITH BROMINATED SIDE CHAINS

Compound[a]	Wave number in reciprocal centimers[b]													
V	1058s		1030s	1015m			950m	915m					732w	725w
III	1055s		1035s	1013m			950m	915m					732w	725w
I	1057s		1031s	1012m			950m	917w			765w			727w
VI	1050s		1030s		983m	973m	912m	912w		886w				
IV	1050s		1035s		985s	975s		910m						
II	1050s		1027s		985s	973m		912w						
IX	1056s		1033s	1015s		973m			901m	875m	777m	748m		
X	1056s		1030s	1015m		971m			901m	875w	777m	747m		
XI	1056s	1040m	1029m	1014m		973m			901m	875w	776w	745m		
VII	1056s		1030s	1010m		974m			900m	875m	777m	750m		
VIII	1055s	1040m	1027m	1012m		972m			900m	873m	776w	750m		

[a] All compounds were 3-acetates: V, 23a-bromotigogenin; III, 23a-bromodiosgenin; I, 5α,6β,23a-tribromodiosgenin; VI, 23b-bromotigogenin; IV, 23b-bromodiosgenin; II, 5α,6β,23b-tribromodiosgenin; IX, 23,23-dibromodiosgenin; X, 23,23-dibromotigogenin; XI, 23,?-dibromotigogenin; VII, 5α,6β,23,23-tetrabromodiosgenin; VIII, 5α,6β,23,?-tetrabromodiosgenin. [b] The relative intensities of the various bands at a concentration of 10 g./liter, 1 mm. cell length are indicated by s (strong), m (medium) and w (weak).

further brominate the monobrominated tigogenin acetate isomers V and VI gave resinous products. Treatment of the various sapogenins dibrominated in the side chain with zinc–acetic acid regenerated the original tigogenin acetate indicating that no major structural changes took place during dibromination.

The infrared spectra of the various brominated sapogenins were invaluable in detecting mixtures and determining the effectiveness of the various procedures used to resolve these mixtures. Table I gives the characteristic frequencies of the diosgenin and tigogenin isomers; Figures 1, 2 give the infrared curves for the various tigogenin isomers. Some general observations on the subject are pertinent. Bromination of the side chain completely changes the typical spiroketal band system.[18] Addition of bromine in the nucleus such as at carbons 5 and 6 had little effect on the infrared spectra as shown by the fact that the spectra of compounds with nuclear and side-chain bromination were similar to those with only side-chain substitution. In addition we noted that the infrared spectra of 5α,6β-dibromodiosgenin acetate closely resembled that of diosgenin acetate. As shown in Table I, the monobrominated 23a- and 23b-isomers readily could be distinguished, the former being characterized by bands absent in the latter (near 1015 and 950 cm.$^{-1}$) and the latter having bands absent in the former (near 985 and 975 cm.$^{-1}$).

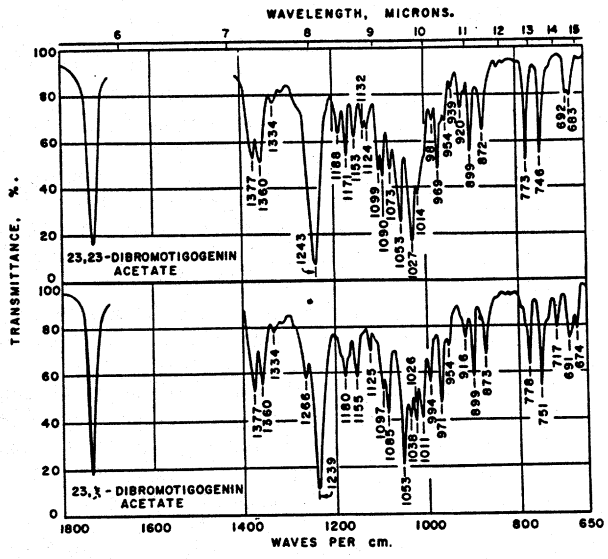


Fig. 1.

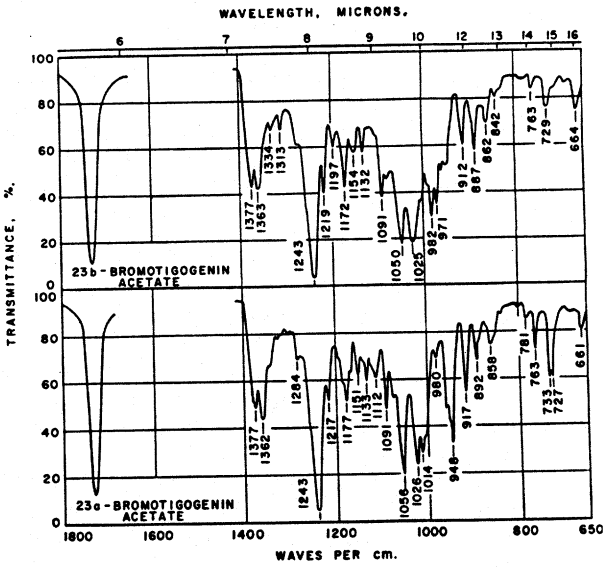


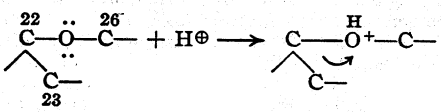
Fig. 2.

The sapogenin isomers containing two bromine atoms in the side chain could be easily differentiated from each other and from the monobrominated compounds. As shown in Table I, this group was characterized by a doublet (probably C–Br) in the region of 745 and 775 cm.$^{-1}$. In the case of the 23,23,-dibromo- group the band near 775 cm.$^{-1}$ was invariably slightly stronger than that at 750 cm.$^{-1}$, whereas the reverse was true in the group designated as 23,?-dibromo. In addition, this latter group had a band absent in the 23,23-dibromo- series near 1040 cm.$^{-1}$.

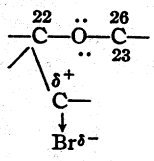
During the course of these investigations, it was noted that the presence of bromine in the side

(18) (a) M. E. Wall, C. R. Eddy, M. L. McClennan and M. E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952); (b) R. N. Jones, E. Katzenellenbogen and K. Dobriner, THIS JOURNAL, **75**, 158 (1953).

chain markedly stabilized the spiroketal moiety against attack by acidic reagents. For example, it is well known that catalytic hydrogenation of sapogenins in acidic media opens ring F with the formation of dihydrosapogenins.[19] A rationalization of this attack on the spiroketal side chain has been given in previous papers of this series[9,20] Under our experimental conditions using acetic acid as a solvent, diosgenin and tigogenin were rapidly hydrogenated in 1–2 hr. Under the same conditions, the side chains of the corresponding 23a- and 23b-bromo analogs were not appreciably affected up to 8 hr., after which time disappearance of bromine and formation of dihydrosapogenins occurred. Another example is the action of phosphorus halides or thionyl chloride on the sapogenin side chain. Djerassi, Ringold and Rosenkranz[21] and Wall and Serota[22] have noted interaction of the sapogenin side chain with such reagents. We have found that monobromination of the spiroketal side chain prevents attack of these reagents on the side chain.[23] We have also noted that 23-bromohecogenin acetate could be successfully converted to $\Delta^{9(11)}$-23-bromohecogenin acetate with selenium dioxide in acetic acid whereas this reagent attacks the side chain of hecogenin acetate.[24] The stabilizing effect of the bromine atom on the spiroketal side chain may be explained as follows. When unbrominated sapogenins are subjected to a proton attack, it seems probable that an oxonium ion is formed



which under favorable conditions may lead to a cleavage of the C_{22}–O– bond. In the case of sapogenins brominated at C_{23}, the halogen produces an inductive effect as



This bring about an electron transmission from C_{22} toward C_{23} and *opposes* the transmission of electrons from C_{22} to the positively charged oxonium ion. The foregoing rationalizes the resistance of brominated sapogenins to catalytic hydrogenation, no reaction occurring until the bromine atom is removed, and the increased stability of brominated sapogenins in general toward attack by Lewis acids.

(19) R. E. Marker, *et al.*, THIS JOURNAL, **69**, 2167 (1947).

(20) M. E. Wall, S. Serota and C. R. Eddy, *ibid.*, **77**, 1230 (1955).

(21) C. Djerassi, H. J. Ringold and G. Rosenkranz, *ibid.*, **73**, 5513 (1951).

(22) M. E. Wall and S. Serota, *ibid.*, **78**, 1747 (1956).

(23) Thus in experiments to be reported in a separate paper we have noted that 23 bromo-rockogenin-3 monoacetate and 23 bromo-11 keto rockogenin-3 monoacetate retained the sapogenin side chain on reaction with reagents such as phosphorus tribromide or thionyl chloride. Unfortunately, the desired replacement of the equatorial 12β-hydroxyl group by halogen (*cf.* E. Borgstrom and T. F. Gallagher, *J. Biol. Chem.*, **177**, 951 (1949)) did not occur. Instead under mild conditions no reaction took place, under more drastic conditions ester formation or ring C/D contraction–expansion took place (*cf.* R. Hirschmann, C. S. Snoddy, Jr., C. F. Hiskey and N. L. Wendler, THIS JOURNAL, **76**, 4013 (1954)).

(24) Selenium dioxide was first used on bromosapogenins by Marker and co-workers[19] and later by Hirschmann, *et al.*, *ibid.*, **75**, 3252 (1953). The former group noted the increased stability of bromosapogenins toward attack by selenium dioxide.

Experimental[25]

5α,6β,23a-Tribromodiosgenin Acetate (I).—18.24 g. of diosgenin acetate (0.04 mole) was dissolved in 2.0 liters of glacial acetic acid. To the solution were added 8 drops of acetic acid saturated with hydrogen bromide. The solution was cooled to 15–20°. A solution of 0.12 mole of bromine in 160 ml. of acetic acid was added dropwise over a period of 30 minutes to the above solution with constant stirring. About 2 hr. after the addition of the bromine, a precipitate was noted in the orange solution. The precipitate was filtered. The filtrate, containing I, was mixed with two volumes of water and the solids filtered. Crystallization from acetone gave 13.5 g. of crude I. Purification was carried out by triturating the product in warm acetic acid and discarding insoluble material. The analytical sample after several crystallizations from acetone was obtained as rods, m.p. 158–162°, $[\alpha]^{25}D$ −108°, infrared spectrum shown in Table I.

Anal. Calcd. for $C_{29}H_{43}O_4Br_3$: Br, 34.53. Found: Br, 34.30.

5α,6β,23b-Tribromodiosgenin Acetate (II).—The precipitate obtained during the bromination described in the preceding section was crystallized from acetone to give 4.9 g. of II. Purification was carried out by trituration with warm acetic acid and discarding *soluble* material. The insoluble fraction was crystallized from acetone to give plates, m.p. 174–176°, $[\alpha]^{25}D$ −112°, principal infrared bands shown in Table I.

Anal. Calcd. for $C_{29}H_{43}O_4Br_3$: Br, 34.53. Found: Br, 34.29.

23a-Bromodiosgenin Acetate (III).—One gram of I was refluxed for 2 hr. with 200 ml. of ethanol containing 0.2 g. of sodium iodide. The solvent was removed *in vacuo* and the residue taken up in ether. The ethereal solution was washed with a dilute sodium thiosulfate solution, then with water, dried over sodium sulfate and the solvent removed. The residue was dissolved in hexane and chromatographed on Florisil. A small fraction was eluted with hexane. The major portion was eluted with benzene. After evaporation of the solvent, the residue was crystallized from acetone–methanol, rods, m.p. 171–175°, $[\alpha]^{25}D$ −111°, chief infrared bands shown in Table I.

Anal. Calcd. for $C_{29}H_{43}O_4Br$: Br, 14.95. Found: Br, 14.72.

23b-Bromodiosgenin Acetate (IV).—One gram of II was treated with sodium iodide as described above and the product chromatographed on Florisil. The main fraction was eluted with hexane. The hexane was evaporated and the residue crystallized from acetone–methanol to give thick plates, m.p. 195–198°, $[\alpha]^{25}D$ −138°, chief infrared bands shown in Table I.

Anal. Calcd. for $C_{29}H_{43}O_4Br$: Br, 14.95. Found: Br, 14.62.

23a-Bromotigogenin Acetate (V). (a) From Tigogenin Acetate.—9.16 g. of tigogenin acetate (0.02 mole) was dissolved in 1.0 liter of glacial acetic acid and treated with 0.03 mole of bromine exactly as described previously under the preparation of I. In this case a precipitate was not observed. The acetic acid solution was poured with stirring into two volumes of water and the solids recovered by filtration. Repeated fractional crystallization from acetone separated two isomers. The separation could be followed by infrared (see Table I, Compounds V and VI) microscopic observation and melting point differences. In this manner pure V, 1.5 g., was obtained in the fractions relatively less soluble in acetone as thick hexagonal plates, m.p. 205–210°, $[\alpha]^{25}D$ −60°.

(25) All melting points were obtained with a Kofler micro melting point apparatus. Optical rotations were determined in chloroform solution, concentration *ca.* 8.0 mg./ml. The infrared spectra were obtained on a Perkin–Elmer Model 21 spectrophotometer using a sodium chloride prism, in carbon bisulfide solution, concentration 10.0 grams per liter. We wish to thank S. Serota for the optical rotation determinations, C. R. Eddy and C. S. Fenske for infrared and Ruth B. Kelley for bromine determinations.

Anal. Calcd. for $C_{29}H_{45}O_4Br$: Br, 14.87. Found: Br, 14.50.

(b) **From Bromodiosgenin Acetate (III).**—0.1 g. of III was dissolved in glacial acetic acid and hydrogenated for five hours at room temperature and three atmospheres in the presence of 0.1 g. of platinum oxide (Adams catalyst). After filtering the catalyst and evaporating the solvent, the residue on crystallization from acetone gave V, m.p. 204–208°. Similar results were obtained by hydrogenating III in the same manner in ether containing 5% acetic acid or ethanol acidified with a few drops of hydrochloric acid. Similar hydrogenation in acetic acid for 15 hr. gave a product free from bromine identified as dihydrotigogenin-3 monoacetate. Tigogenin acetate was converted to the same product in 2 hr. under the above conditions.

23b-Bromotigogenin Acetate (VI). (a) From Tigogenin Acetate.—The *more soluble* acetone fractions, described under V above, contained VI, 1.2 g., rods, m.p. 220–230°, $[\alpha]^{25}D$ −81°.

Anal. Calcd. for $C_{29}H_{45}O_4Br$: Br, 14.87. Found: Br, 14.80.

(b) **From 23b-Bromodiosgenin Acetate (IV).**—Catalytic hydrogenation of IV in the manner described under V, part (b), gave VI, m.p. 222–229°.

5α,6β,23,23-Tetrabromodiosgenin Acetate (VII).—Diosgenin acetate (9.12 g., 0.02 mole) was dissolved in one liter of glacial acetic acid. To this solution 0.08 mole of bromine in 100 ml. of acetic acid was added in the usual manner. No precipitation occurred. After standing 3 hr. at 15–20°, the solution was allowed to come to room temperature and then to stand for 72 hr. The acetic acid solution was poured into excess water precipitating the brominated sapogenin and the solids collected by filtration. They were taken up in hexane, acetic acid neutralized with sodium bicarbonate solution and the hexane dried over anhydrous sodium sulfate. Chromatography on Florisil gave 0.75 g. of crystalline product on hexane elution and 1.0 g. of product on benzene elution. Elution with chloroform and alcohol accounted for the bulk of the weight. These products were dark resins. Acetone crystallization of the solids from the hexane elution gave VII, 0.3 g. prisms, m.p. 177–180°, $[\alpha]^{25}D$ −105°, chief infrared bands shown in Table I.

Anal. Calcd. for $C_{29}H_{42}O_4Br_4$: Br, 41.34. Found: Br, 41.92.

5α,6β,23,?-Tetrabromodiosgenin Acetate (VIII).—Acetone crystallization of the *benzene* eluate described above gave VIII, 0.2 g., rods, m.p. 188–191°, $[\alpha]^{25}D$ −43°, chief infrared bands shown in Table I.

Anal. Calcd. for $C_{29}H_{42}O_4Br_4$: Br, 41.34. Found: Br, 40.92.

23,23-Dibromodiosgenin Acetate (IX).—One-tenth gram of VII or VIII was refluxed with sodium iodide in ethanol as described previously. After acetone crystallization only IX was obtained in each case, m.p. 193–198°, $[\alpha]^{25}D$ −94°, chief infrared bands shown in Table I.

Anal. Calcd. for $C_{29}H_{42}O_4Br_2$: Br, 25.97. Found: Br, 25.77.

23,23-Dibromotigogenin Acetate (X).—Tigogenin acetate (9.16 g., 0.02 mole) in one liter of glacial acetic acid was brominated with 0.06 mole of bromine as described under the preparation of VII with the exception that the product was held at room temperature for 24 hr. . The brominated sapogenin was transferred to hexane as under VII and then chromatographed on silica gel. Elution with hexane–benzene, 1:1, gave 6.0 g. of semicrystalline product; elution with benzene gave 1.0 g. of crystals mixed with resin, further elution with more polar solvents yielded only dark resins. Methanol crystallization of the residue from the hexane–benzene eluates gave X, 0.5 g., m.p. 199–200°, $[\alpha]^{25}D$ −68°, chief infrared bands shown in Table I.

Anal. Calcd. for $C_{29}H_{44}O_4Br_2$: Br, 25.89. Found: Br, 25.60.

X was also obtained by catalytic hydrogenation of IX using platinum oxide catalyst and ether containing 5% acetic acid as the solvent.

23,?-Dibromotigogenin Acetate (XI).—The *benzene* eluate obtained in preparation of X above was evaporated and the residue crystallized from methanol repeatedly to yield XI, 0.06 g., m.p. 178–182°, $[\alpha]^{25}D$ −40°, chief infrared bands shown in Table I.

Anal. Calcd. for $C_{29}H_{44}O_4Br_2$: Br, 25.89. Found: Br, 25.42.

23,23-Dibromotigogenin acetate (X), 0.025 g., and 23,?-dibromotigogenin acetate (XI), 0.015 g., were refluxed with sodium iodide as described under III. The infrared spectra of the crystalline residues showed that X was unchanged and that XI was completely converted to X.

Compounds X and XI were dissolved in acetic acid and heated in the presence of zinc dust. Infrared examination of the crystalline reaction products indicated that each compound had been converted to tigogenin acetate.

Equilibration Experiments.—(a) 23a-Bromodiosgenin acetate (III) (0.2 g.), in 100 ml. of ethanol containing 1 ml. of 48% hydrobromic acid was refluxed 12 hr. The product obtained by ethereal extraction in the usual manner was acetylated and found to be unchanged III. Similar treatment of 23b-bromodiosgenin acetate (IV), yielded unchanged starting material.

(b) 23a-Bromotigogenin acetate (V) (0.1 g.), in 30 ml. of anhydrous ether containing 3 ml. of acetic acid saturated with hydrogen bromide (approximately 38% solution) was allowed to stand overnight. After the usual work-up, only unchanged V was isolated. Similar treatment of 23b-bromotigogenin acetate (VI), 23,23-dibromotigogenin acetate (X) and 23,?-dibromotigogenin acetate (XI) gave, respectively, unchanged VI, X and XI.

Deuteration of Sapogenins.—Deuterium acetate was prepared by mixing acetic anhydride with the appropriate quantity of deuterium oxide (98%).

(a) 23a-Bromodiosgenin acetate (III) (0.5 g.), was refluxed 5 hr. with 75 ml. of deuterium acetate in the presence of 10.0 g. of zinc dust. The residue obtained after filtering the zinc and evaporating the solvent was crystallized from methanol and gave 0.3 g. of long needles, m.p. 196–199°, $[\alpha]^{25}D$ −117°. The infrared spectrum of the product, which we regard as 23,23-deutero-diosgenin acetate (XII), showed marked deviations from that of diosgenin acetate. In particular the strong band near 900 cm.$^{-1}$ and the weaker band near 865 cm.$^{-1}$, typical of the 25D series, were absent.

Similar treatment of 23b-bromodiosgenin acetate (IV) gave a compound identical to XII, the identity being verified by comparison of infrared spectra and X-ray diffraction patterns.

(b) Diosgenin acetate (0.1 g.), was refluxed 2 hr. with deuterium acetate.[15,16] The product isolated in the usual manner was XII.